Reference : *Biol. Bull.* **159**: 102–116. (August, 1980)

DEVELOPMENT OF THE CILIATURE PATTERN ON THE EMBRYO OF THE SQUID *LOLIGO PEALEI*: A SCANNING ELECTRON MICROSCOPE STUDY

JOHN M. ARNOLD AND LOIS D. WILLIAMS-ARNOLD

Kewalo Marine Laboratory, Pacific Biomedical Research Center, 41 Ahui St., Honolulu, Hawaii 96813; and Marine Biological Laboratory, Woods Hole, Massachusetts, 02543

One feature of embryonic development that seems almost universal is the appearance of ciliated cells on the epithelium of developing embryos. These ciliated cells may be scattered over the embryonic surface or may exist in patterns specific enough to have taxonomic or evolutionary significance. Although there is great interest in the biochemical basis of movement in cilia and flagella, there seems to be little current interest in the significance to the embryo of the motion generated by ciliated cells. This paper describes the development of the ciliature pattern on the embryos of *Loligo pealei* and speculates on the function and significance of the various types of ciliated cells found at different times during development.

Relatively few scanning electron microscope (SEM) studies have been made on the patterns of ciliature of developing embryos and most of these have been done on amphibians. Kessel *et al.* (1974) found in *Rana pipiens* that ciliated cells appeared in the future epidermis during the neural plate stage and that the number of ciliated cells increased greatly during development of the neural folds. By the tailbud stage ciliated cells were widely distributed over the entire embryonic surface but in post-hatching larvae (Stage 24) the cilia began to regress, apparently by resorption. They speculated the ciliated cells functioned in embryonic movement, facilitated respiration, and were important in the movement of mucus across the embryo.

Billett and Courtenay (1973) and Landstrom (1977) have examined development of the ciliature of *Ambystoma mexicanum* embryos. Ciliated cells first appear at the one-somite stage. By the time four to eight somites are formed, one in three surface cells on the flank are ciliated. These ciliated cells are always separated from each other by non-ciliated cells. There is a weak anterior-posterior gradient in number of the ciliated cells but some ciliated cells are present on every surface region. Since the ciliated cells appear first in slight depressions or pits, Billett and Courtenay (1973) speculate that these cells may be moving up from below the epidermal layer. Smith *et al.* (1976) treated *Xenopus* embryos with lithium to produce exogastrulae and found that appearance of ciliated cells was delayed, while Grunz *et al.* (1975) found vegetalizing agents inhibited formation of ciliated cells in isolated *Triturus* ectoderm.

Bergquist *et al.* (1977) found "club-footed" cilia in several species of *Demospongiae*. These cilia have terminal expansions several times the diameter of the ciliary shaft. The expansions are sometimes flattened into paddles and contain irregular membranous vesicles and dense material in addition to a typical axoneme. They did not assign a special function to these "club-footed" cilia but did speculate that such an expansion could cause more efficient movement of the larval sponges.

Sundermann-Meister (1978), using light microscopy, described a new type of ciliated cell from late embryos and juvenile *Loligo vulgaris*. Cilia of these cells are fused into a single common rod. These rods project from surfaces of the fin, mantle, funnel apparatus, head, arms, and suckers. This cilia-rod does not appear to beat and may be a mechanoreceptor.

In this paper we describe four different types of ciliated cells and discuss their function(s) in relationship to the embryonic metabolism.

Materials and Methods

Embryos were obtained from adult *Loligo pealei* using methods described by Arnold (1962); the embryonic stages referred to are those of Arnold (1965). For scanning electron microscopy, embryos were fixed at room temperature in 2% glutaraldehyde in sea water buffered in pH 7.4 with collidine-HCl for 30–40 min. After a 30 min rinse in collidine-HCl buffered sea water, the embryos were post-fixed in 1% OsO_4 in sea water adjusted to pH 7.4 with collidine for 30–45 min. The embryos were then dehydrated through a graded series of acetone, and critical-point dried with CO_2. They were then mounted on metal stubs and coated with gold in a vacuum evaporator with a rotating stage or with a sputterer. Some cracking of the surface occurred because of the massive amount of yolk in the embryo and the hemal sinuses.

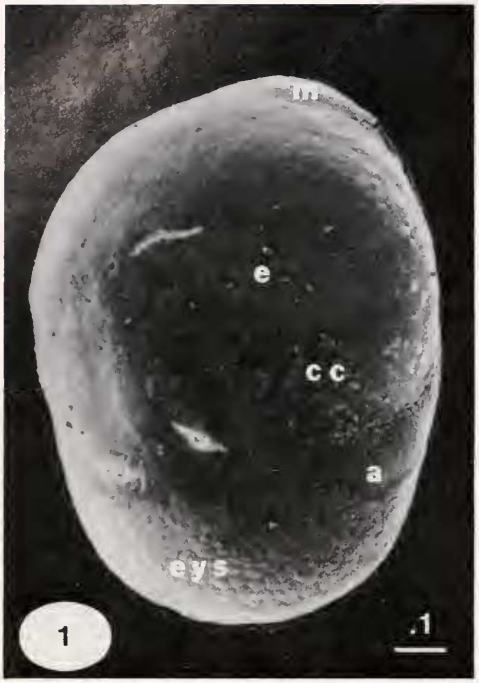
Fixation for transmission electron microscopy was done in 1% OsO_4 with Palade's buffer at pH 6.8–7.0 for 20–30 min at room temperature. The embryos were dehydrated with a graded ethanol series and embedded in Luft (1961) Epon.

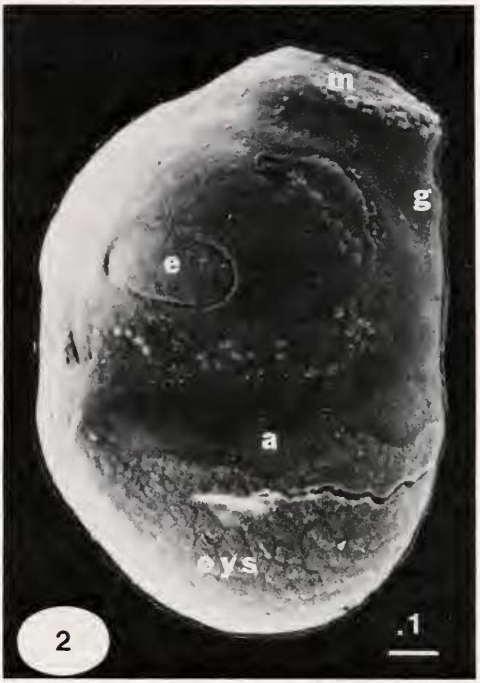
Because many of the low magnification micrographs had backgrounds which distracted from and confused the image of the embryo itself, in several instances (Figs. 1, 2, 7, 8, 11–16, 20–22, 24, 25, and 27) the image of the embryo was carefully cut out of the micrograph and mounted on an artificial background of black photographic paper.

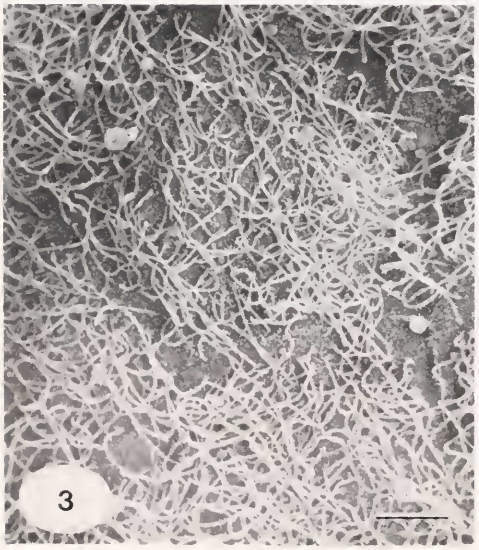
Measuring the speed and direction of particles moved by the ciliated cells in living embryos was found impractical because of heterogeneous distribution of some of the ciliated cells. It was possible, however, to plot the general direction of particle flow. This is expressed with arrows on some micrographs. On the external yolk sac where the ciliated cells are quite uniform, it was possible to measure particle movement as 1.18 mm/sec. But this cannot be compared to the other types of ciliated cells.

Results

Ciliated cells first appear at the same time as or slightly before the organ primordia (Stage 17). They are first evident on the future external yolk sac in a pavement-like arrangement of polygonal flattened cells on the lower $\frac{1}{2}$–$\frac{1}{3}$ of the embryo (Figs. 1 and 2). All cells of the external yolk sac are ciliated with no intervening glandular or other epithelial cells (Fig. 3). The margin of each cell has an area of variable width free of cilia. The cell surface margin is irregular and not covered with microvilli or other surface protrusions. Directly beneath the cells of the external yolk sac, a large hemal space develops. For a time, this is the primary circulatory and respiratory organ (Arnold, 1971; Arnold and Williams-Arnold, 1976). As the external yolk sac pulses, the individual epithelial cells change shape by stretching or contracting. The individual cilia do not appear







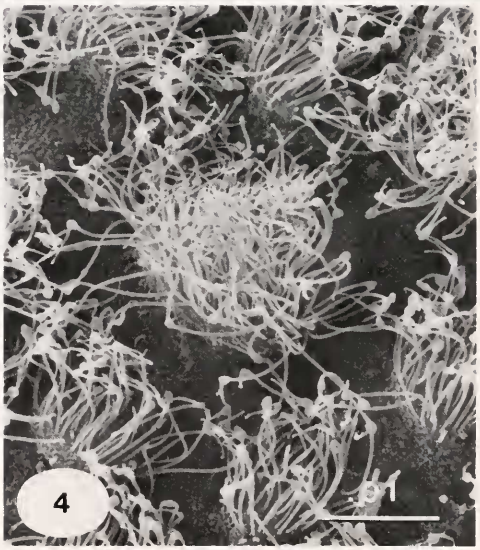
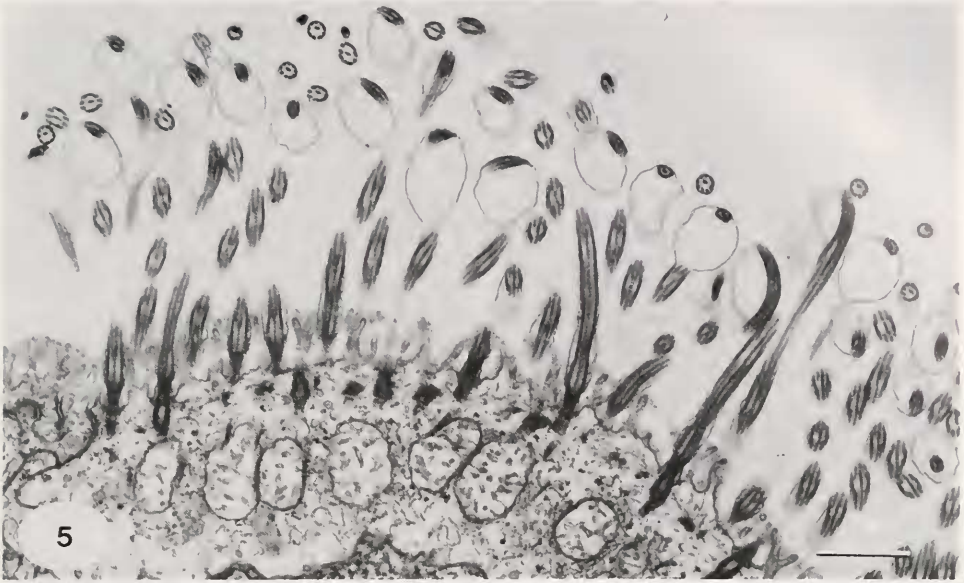
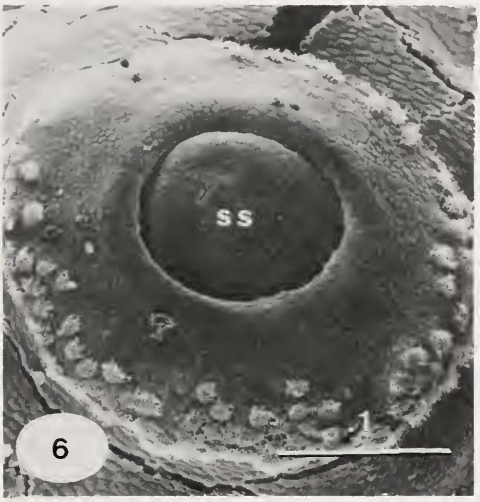


FIGURE 1. Early Stage 18 viewed from the side. The external yolk sac (eys) occupies about ¾ of this embryo and is covered with flattened ciliated cells. The mantle (m), eye (e), and arm (a) primordia are evident. Ciliated cells (cc) are present below and to the right of the eye. Except where different notations are used, all magnification bars are in mm.

FIGURE 2. Stage 19 viewed from the side. The external yolk sac (eys) abuts directly on the arm region and the individual arm primordia (a) are evident. The eye (e) has begun to form the optic vesicle and one gill primordium (g) is visible. The mantle (m) has paddle-type ciliated cells on the future ventral margin.





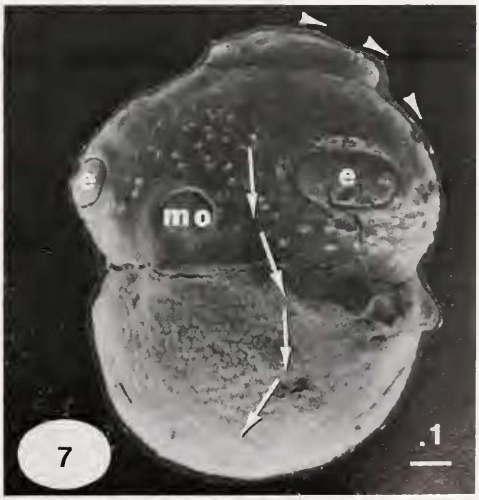


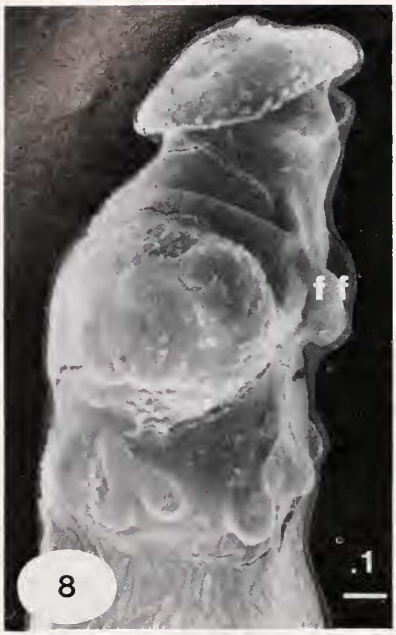
FIGURE 3. Ciliated cells of the external yolk sac at Stage 20. The cells are polygonal in outline and are covered with cilia which appear to beat in an uncoordinated but unidirectional fashion.

FIGURE 4. Paddle-type ciliated cells on the mantle of the Stage 25 embryo. These cells are almost invariably separated from each other by other cells of the epithelium. The individual cilia are expanded into flattened "paddles" or, more infrequently, spheroidal knobs apically or subapically. Although the beat is unidirectional, it is not synchronous.

FIGURE 5. Section of one paddle-type ciliated cell. The paddles are composed of an expanded portion of the cilia membrane, which forms an electron transparent vesicle. The axoneme bends sharply within the paddle.

FIGURE 6. Mantle of a Stage 20 embryo during closure of the shell sac (ss). Paddle-type ciliated cells have developed on the future ventral surface (at magnification bar) and sides of the edge of the mantle.

FIGURE 7. Future dorsal surface of the Stage 20 embryo. The number of paddle-type ciliated cells has increased around the eye primordium (e) and above the mouth (mo). The arrows indicate the direction of flow of the chorionic fluid.



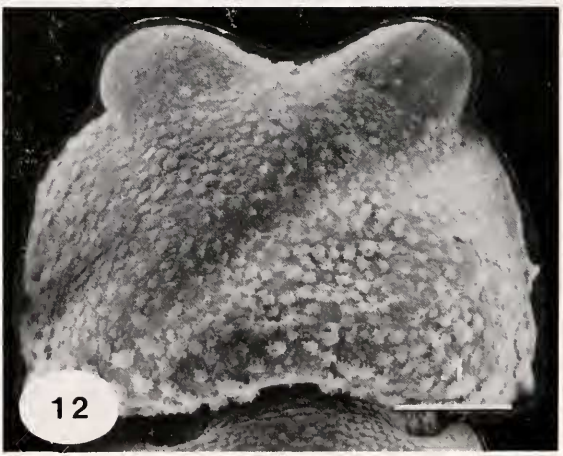
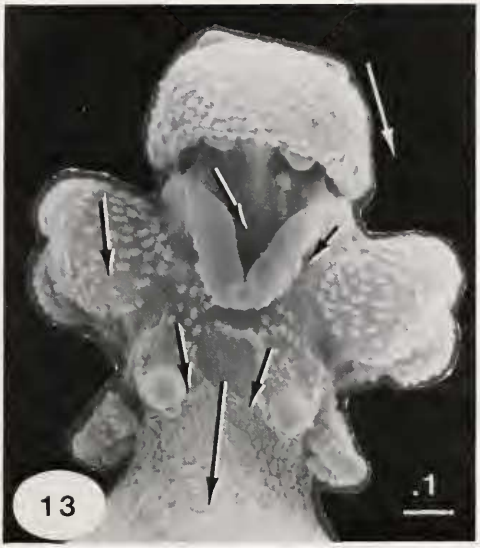
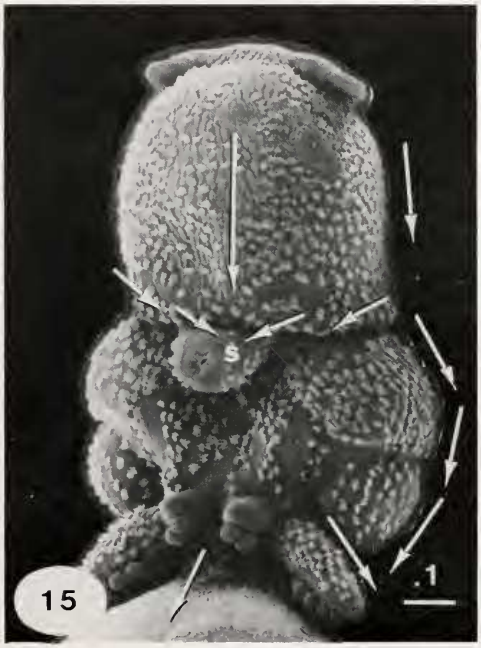


FIGURE 8. Lateral view of a Stage 22 embryo. The external yolk sac has elongated along with the embryo, and cells in the yolk stalk region have changed from polygonal to elongate. A few paddle-type cilia occur on the arms and distal portion of the funnel folds (ff). The number of ciliated cells on the optic stalk has increased.

FIGURE 9. Paddle-type ciliated cells have increased in number and are more or less evenly distributed on the future ventral mantle. The gill primordia (g) and proximal portions of the funnel complex remain unciliated.





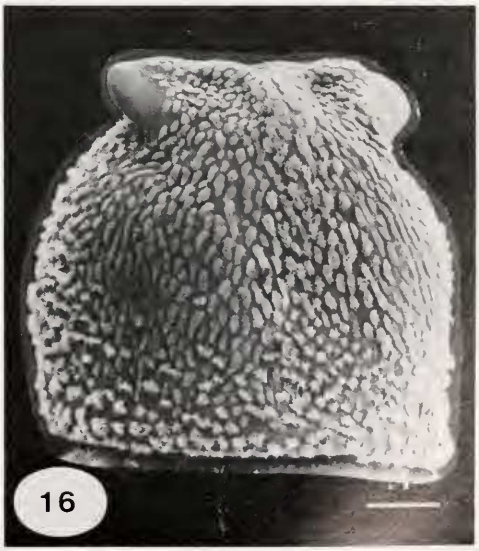


FIGURE 10. The dorsal portion of the mantle is becoming ciliated by Stage 22, although the future dorsal midline is last to develop ciliated cells. The fin primordia (f) do not have any cilia on them.

FIGURE 11–13. The number of paddle-type cilia on the mantle has continued to increase and the pattern seems random. Parts of the eye stalk and and some future ventral areas remain unciliated. The arrows indicate the direction of fluid flow. See text for details.

FIGURES 14–16. At Stage 26, there are regions of the head which lack ciliated cells, notably above the eye and on the future dorsal head. A major change has occurred on the mantle with the appearance of "uniform-type" ciliated cells. These cells tend to form lines in which all the cilia have unidirectional and synchronous beat. (s = siphon). See text for details. On Figure 15 the arrows indicate the direction of movement of the chorionic fluid.

to have any unusual features. In SEM there is no evidence of synchrony of beat, but in living embryos, the cilia beat toward the vegetal pole of the embryo.

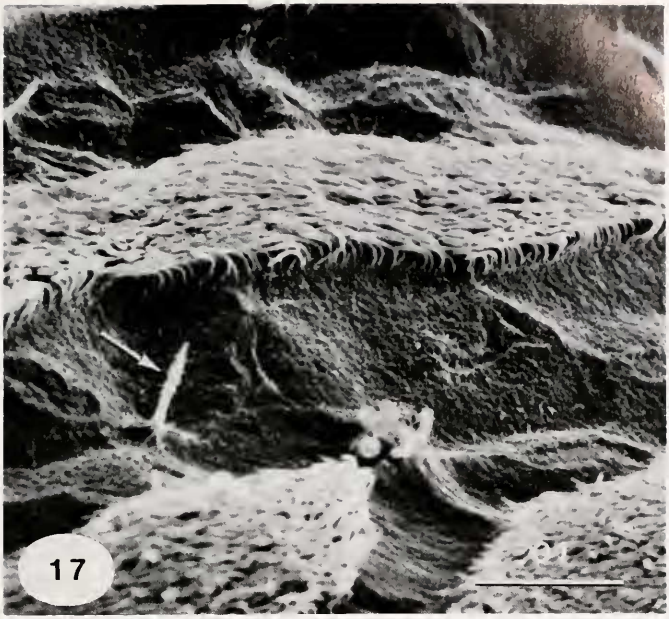
A second type of ciliated cell appears at about Stages 17 and 18, first on the ventral and anterior edge of the eye primordia, then on the future ventral margin of the mantle (Figs. 1 and 2). These cells are large, flattened, and separated from each other by non-ciliated neighboring cells (Fig. 4). These cilia have a unidirectional but not metachronic beat. They are larger than those on the external yolk sac and have an apical or subapical expansion three to five times the diameter of the rest of the cilium (Fig. 4). In the scanning microscope, this expanded portion frequently appears flattened into a paddle shape, although spheroidal expansions are also occasionally seen. Because of this morphology, they will be referred to as "paddle-type cilia." If the expansion is subapical, the tip of the cilium frequently emerges at an acute angle to the rest of the shaft. Transmission electron micrographs show the expanded portion to be a bleb of "empty" membrane at a bend in the axoneme. The axoneme has the typical "9 + 2" morphology (Fig. 5). In living cells, the paddle appears passive and follows the beat of the cilium. When seen on the return stroke with phase-contrast microscopy, the plane of the paddles is close to and parallel with the cell surface. On the power stroke, it is perpendicular to the angle of thrust.

At Stage 20, the paddle-type ciliated cells are on the sides of the mantle primordium, and the number of ciliated cells and area covered on the eye primordium has increased. They also appear above the mouth primordium (Figs. 6 and 7).

At Stage 22 (Figs. 8–10), the paddle-type ciliated cells occur on the top of the optic stalk and on the future anterior surface of the optic vesicle, while the future posterior of the optic vesicle is unciliated. The mantle has expanded in area and turned downward to begin to cover the developing mantle cavity and the number of ciliated cells has increased accordingly. Most ciliated cells still occur singly, but occasionally two cells are in contact (Fig. 9). The fin primordia remain devoid of ciliated cells. The future dorsal region of the mantle still is temporarily devoid of ciliated cells (Fig. 10). A few paddle-type ciliated cells have appeared on the proximal portion of the arms and on the outer distal end of the funnel primordium (Fig. 8; *cf.* Fig. 11).

The embryo elongates as it grows so that the external yolk sac is attached to the embryonic region proper by an ever-narrowing yolk stalk (Fig. 8). This also causes a change in shape of the individual cells, but the direction of beat apparently is unaffected.

The distribution of cilia on the mantle becomes more uniform as the mantle grows over the future mantle cavity (Stage 23, Figs. 11–13). The edge of the mantle has a fairly continuous line of ciliated cells, and in some instances these cells appear larger with longer individual cilia. The fins do not have ciliated cells except at the margin between them and the base of the future dorsal surface (Fig. 12). The number of paddle-type ciliated cells on the body surface has increased but the organs or parts of the organs which will become incorporated into the mantle cavity remain unciliated (*e.g.*, gills, anal papilla, part of the funnel folds; Fig. 13). The optic stalk has a somewhat uniform distribution of paddle-type ciliated cells except on the future posterior surface of the eye and in a band which lies between the optic stalk proper (Fig. 11). Later in embryonic development, this region gives rise to tissue which covers the optic vesicle and forms the definitive cornea. The tips of the arms and the developing suckers also lack ciliated cells.



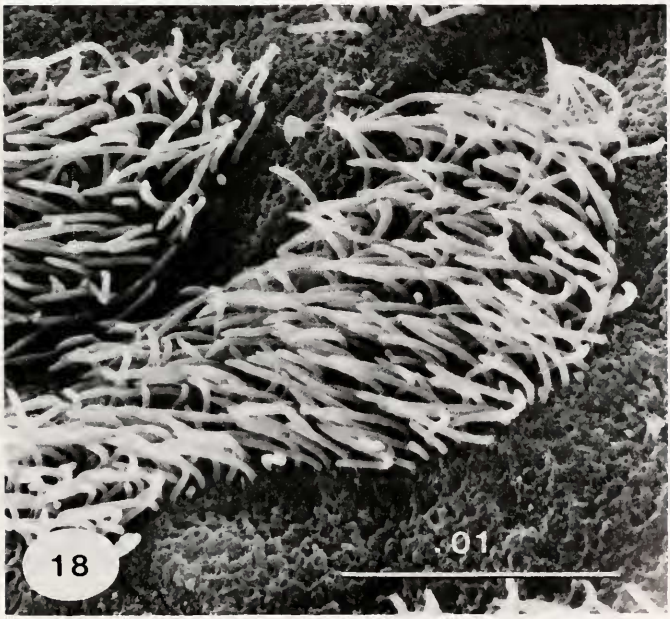
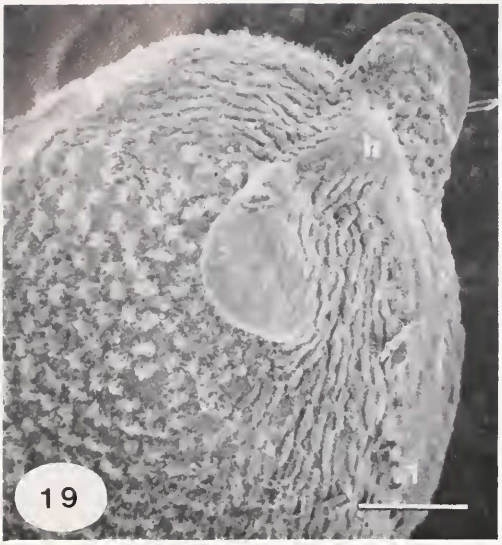
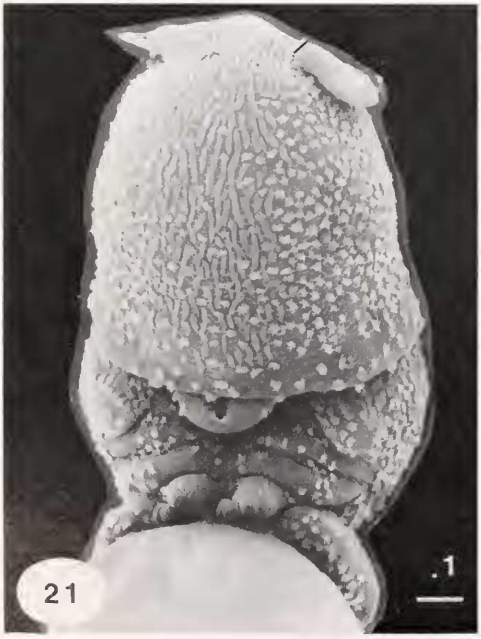


FIGURE 17. The uniform-type ciliated cells beat in a metachronic wave that is synchronized along several aligned cells. The projection in the lower left (arrow) may be a mechanoreceptor.

FIGURE 18. In this developing uniform-type ciliated cell, cilia of several lengths can be seen and the beat, although unidirectional, is only partially synchronous. These cilia are of uniform diameter and end in a blunt taper.

By Stage 26 (Figs. 14–16), the pattern of cilia on the head of the Stage 23 embryo has changed slightly. This includes an increase in the ciliated cells and two lines of paddle-type ciliated cells running from the base of the median pair





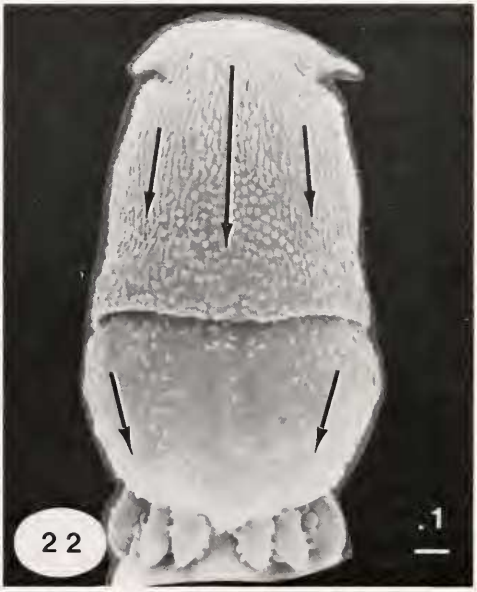


FIGURE 19. The lines of uniform ciliated cells radiate from the developing Hoyle organ (h) at Stage 28. The chorionic fluid is circulated from the future posterior toward the edge of the mantle.

FIGURES 20–22. At Stage 27 the edge of the mantle has a band with relatively few ciliated cells and the lines of uniform-type cilia are more prominent on the future dorsal and future ventral surfaces. On Figure 22, the arrows indicate the general direction of movement of the chorionic fluid.

of arms back toward the optic stalk on the anterior dorsal head (Fig. 14). Ciliated cells are approximately evenly distributed on the posterior dorsal head. The ventral surface of the head is uniformly covered with ciliated cells except between

the future ventral-most arms, in a small region over the otocysts and at the junction of the optic lobe region of the optic stalk and the optic vesicle (Fig. 15).

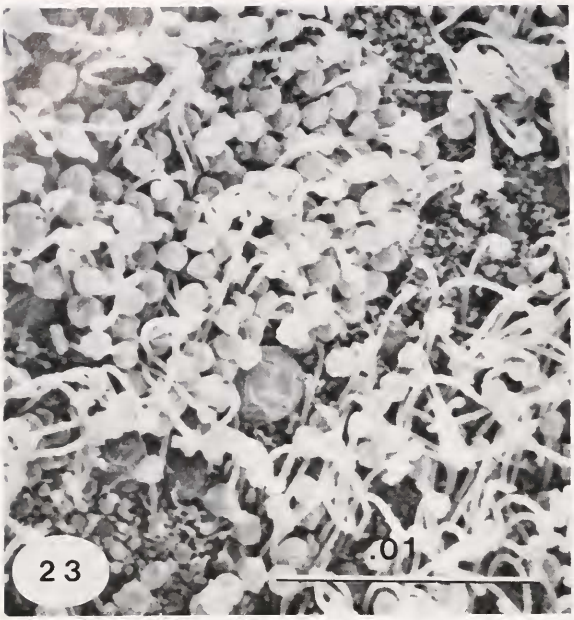
The mantle pattern of ciliature has undergone extensive changes by Stage 28 and a different type of ciliated cell has appeared amid the uniformly distributed paddle-type ciliated cells (Figs. 14–16). These cells are elongate on the anterior-posterior embryonic axis and show a strong tendency to form anterior-posterior contact with each other. The cilia are uniform in diameter and taper to a point without an expanded region (Figs. 17 and 18), and will be referred to here as "uniform-type ciliated cells." These cilia beat in a metachronic wave continuing across the cell borders. The cilia on these cells occur close to the margin of the cells. As many as seven cells occur in such an alignment, with groups of four and five common. These lines rarely branch or fork and if they do, it is with one cell. The lines of cells radiate from the future site of the Hoyle organ (hatching gland) which is situated between the fins (Fig. 19). The cilia of the cells do not grow out of the cell simultaneously: In young cells several lengths of cilia can be found and the beat pattern seems irregular (Fig. 18).

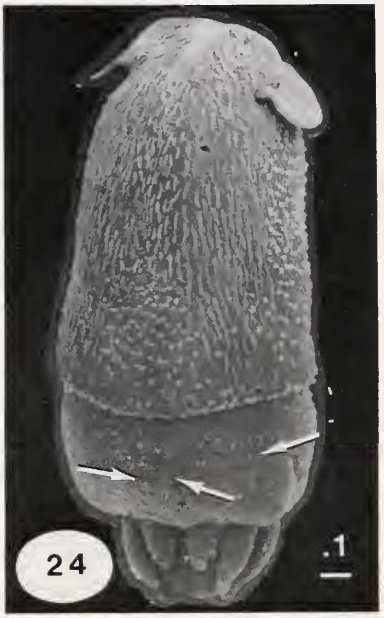
At Stage 27, the mantle elongation has increased so that a band relatively sparse in ciliated cells occurs behind its anterior margin (Figs. 20–22). The ciliature pattern is unchanged except that the ciliated cell-free area of the dorsal head has increased. Aligned groups of uniform-type ciliated cells of the mantle occur in one ventral medial patch and in a "V" on the dorsal surface (Figs. 21 and 22). Between the arms of the "V" and on the sides of the mantle are extensive areas of paddle-type ciliated cells.

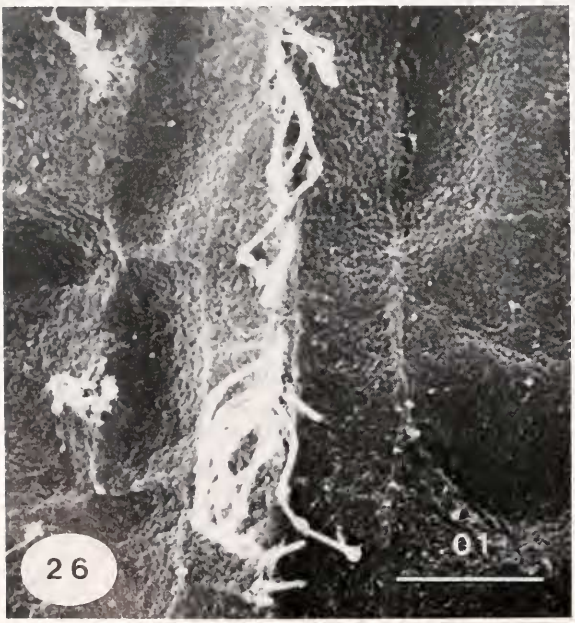
As the embryo grows, the external yolk sac decreases by digestion of yolk and by transfer of its contents into the developing internal yolk sacs. The circumoral musculature constricts the hemal space in the arm region and pulsation of the yolk sac decreases. The cilia of the yolk sac also shorten and become irregular in diameter. They still beat, however, because particles in the chorionic fluid or sea water sweep along the surface of the external yolk sac. High magnification reveals that the apical and subapical portion of these cilia have expanded to flattened discs similar to those of the "paddle-type cilia." Furthermore, these discs frequently have double ridges at their edges, each of axoneme diameter. (Fig. 23).

Before hatching (Stage 29), the external yolk sac continues to decrease in size and the embryo to elongate, causing the yolk-sac cells to become rounded and have a smaller external area. The ciliature pattern on the mantle remains similar to that of the Stage 27 embryo (*cf.* Figs. 21 and 22 with Figs. 24 and 25), but on the dorsal surface of the head, a fourth type of ciliated cell appears (Figs. 24–26). This type occurs as two to four lines of "single-file" ciliated cells running along a central head crest (Fig. 26). Two such rows in the central region of the head are common, but four or occasionally three lines occur. Side-to-side synchronous beat appears along the length of the "single-file" ciliated cell. Exceptionally, the tips of these cilia are apically or subapically expanded; mostly they end in a short tapered tip.

After hatching (Stage 30), an abrupt change occurs in the epithelium of the mantle. Within 24–36 hr, the cells of the mantle begin to slough off and are released into the surrounding sea water as individuals or in small groups (Figs. 27 and 28). The surrounding glandular elements may also be shed. Large gaps appear between the cells bridged by many fine branching extensions of surface material (Figs. 28 and 29).







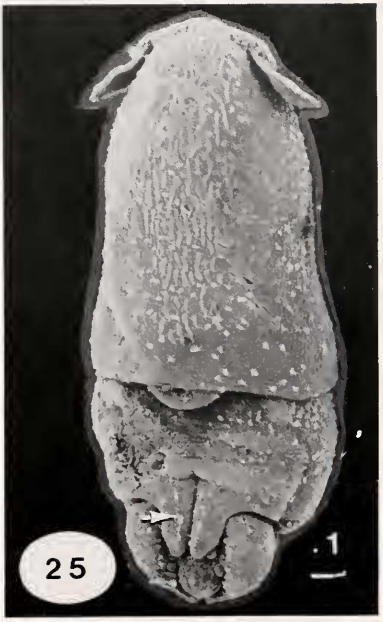
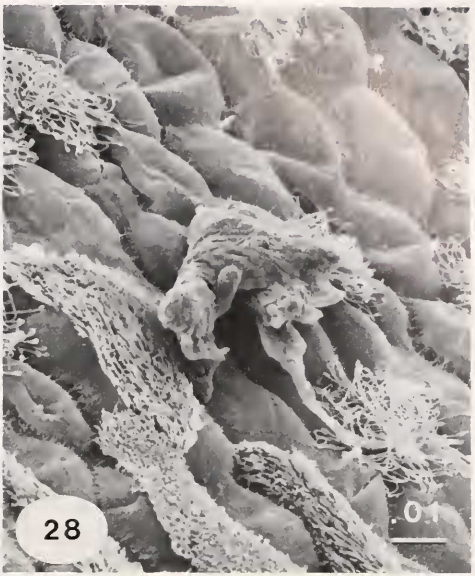


FIGURE 23. After heart and gills assume the respiratory and circulatory functions of the external yolk sac, the sac stops pulsating and the cilia shorten and develop disc-like paddles, possibly by coiling of the axoneme. They continue to beat.

FIGURES 24 and 25. At Stage 29, the ciliature pattern on the mantle has changed little from Stage 27, but "single-file" ciliated cells have appeared on the head (arrows).

FIGURE 26. Higher magnification of the single-file ciliated cells show the cilia arise from a central crest of cells and beat in a lateral wave.



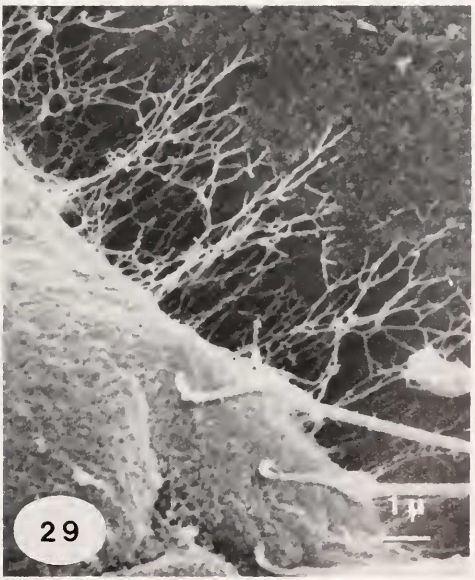


FIGURE 27. After hatching, the ciliated cells of the mantle are shed within 24–36 hr. The ciliated cells of the head are lost later.

FIGURE 28. During shedding, cells separate from each other in longitudinal rows and are lost singly or in small groups. Mucus cells are also probably lost.

FIGURE 29. As the cells of the mantle epithelium pull apart, a meshwork of branching strands which intertwine with cilia becomes apparent. Frequently, cilia can be seen sticking to the surface of non-ciliated cells.

The head ciliature does not similarly change during the 2–3 days we have been able to keep newly hatched juveniles in good health (Fig. 27). Presumably it does so later. The external yolk sac usually is dropped and degenerates soon after hatching. Its cilia are active, however, for a short time after it is cast aside.

Occasionally, non-moving bundles of cilia are observed in the mantle (Fig. 17), or more commonly on the aboral surface of the arms. Presumably these are the mechanoreceptors described by Sundermann-Meister (1978).

Discussion

The question raised by these observations is: Why do three different types of ciliated cells exist to perform similar functions? The time of appearance and placement on the embryo may provide some insight about the function of each type; still, all the questions cannot be answered. The function of the fourth type, the "single-file" cilia, is unknown to us.

Cilia of the external yolk sac arise first. Since the external yolk sac is an embryonic digestive, circulatory, and respiratory organ, its ciliated cells are most probably associated with respiration. The hemal space of the external yolk sac is separated from the chorionic fluid by a thin layer of flexible pavement-like cells. Cilia in this position prevent localized accumulations of carbon dioxide (or excretory products) and facilitate respiratory exchange. Cilia of these cells have a directional beat, but individual cilia do not beat synchronously and unlike amphibian embryos (Kessel *et al.,* 1974; Billett and Courtenay, 1973) the ciliated cells are in contact with each other at their margins. Collectively, these ciliated cells must be quite effective in circulating the chorionic fluid, which in turn is able to exchange metabolic substances through the chorion. Their partial structural degeneration when the gills and branchial hearts take over the respiration and circulation of the developing embryo suggests further that they function primarily in respiratory exchange. Apparently, the development of a disk-like paddle on or near the tips of the cilia later in development is related to the coiling of the axonemes in each individual cilium.

The function of the paddle-type cilia is more obscure. The development of a flattened expanded tip or subapical portion could increase efficiency of the effective stroke. By being flattened, the expanded tip would create little drag in the recovery stroke if the plane of flattening is perpendicular to the plane of bending. The sharp bending of the axoneme where it passes through the expanded portion could be structurally related to strengthening the "paddle" during the effective stroke if the curvature of the band is oriented into the plane of the effective stroke. Observations with phase contrast microscopy confirm this is likely.

Paddle-type ciliated cells always appear isolated from other ciliated cells and first appear on the organ primordia. They are particularly prominent and active. Their placement on the eyes, head, arms, and early mantle suggest that they generally circulate the chorionic fluid past the embryo but also prevent the embryo from directly contacting and possibly attaching to the inside of the chorion. Isolated, non-ciliated embryonic cells do stick to the chorion. Their position on the embryo would also facilitate "cutaneous" respiration. They do not beat synchronously, suggesting autonomous control. The uniform-type ciliated cells have the appearance usually associated with ciliated cells on other tissues and embryos. Their most striking feature is that they have a metachronic beat and synchrony extends from one cell to another in a continuous wave. The alignment and synchrony of the beat of these ciliated cells seems quite effective in moving large volumes of fluid. Since these ciliated cells appear about the same time (Stage 26) as mantle contraction begins and the gills and branchial hearts begin to function, they could increase chorionic fluid circulation. In fact, older embryos

continuously tumble inside the ever-expanding chorion. Dechorionated embryos observed in a dish of sea water continuously move posteriorly along the bottom of the dish. Therefore, these rows of aligned synchronous ciliated cells possibly function in enhancing the circulation of the chorionic fluid and movement of the older embryo. This is advantageous to the embryo for respiration and excretion.

The detailed cause(s) of the post-hatching sloughing of the skin of the newly hatched juvenile are unknown. But the phenomenon obviously is related to the breaking of cell-to-cell contacts and cells are lost either as individuals or in small groups. The filamentous material that temporarily connects the cells as they slough is probably the remnant of intercellular substances, since the cell membrane does not appear to be involved. We assume that this sloughing is followed by the development of the slimy skin typical of cephalopods. Because of the difficulties in rearing juvenile squid in the laboratory, we were unable to collect data on this.

The single-file cilia do move, so it would seem unlikely that they function as mechanoreceptors; and since they are not in an enclosed pit, it is unlikely that they are chemosensory. They could possibly function to keep the head free of detritus, but there is never much particulate material in the chorionic fluid and since they are in a single row, it seems unlikely that they could generate much force for this purpose. However, they may move mucus produced by the gland cells of the skin on the head. How long these cilia persist and their ultimate fate is unknown.

The developmental significance of morphologically different ciliated cell types is puzzling. Because they are functionally different and appear at different times during development and on different organs, there must be adequate selection pressure to preserve their separate phenotypes. This is striking since on other embryos, only one recognized motile ciliated cell type must provide all the surface ciliary functions for complete embryonic development. Perhaps these differences between vertebrate embryos and cephalopod embryos reflect their separate evolutionary histories.

The authors wish to thank Drs. Lewis Tilney and Michael Hadfield for reading this manuscript and Mrs. Frances Okimoto and Mrs. Charlotte Daspit for preparing the typescript. This work was supported by grant #PCM 7619301 from the National Science Foundation and the *Teuthis obscura* fund. Dr. Virginia Peters taught us many of the techniques used here.

Summary

1. Four types of beating ciliated cells, which appear at different times during embryonic development of the squid *Loligo pealei*, are described.

2. The ciliated cells of the external yolk sac first appear during or at onset of organogenesis, as polygonal flattened cells in a pavement-like arrangement. The cilia of these cells are uniform in diameter and end in a blunt or slightly tapered tip. Their beat is asynchronous and uncoordinated, but unidirectional toward the vegetal pole. A large hemal space develops between these cells and the yolk syncytium of the external yolk sac. In later stages of development, when primary respiratory function is assumed by the gills (and probably the skin), the

cilia on those cells develop a flattened paddle at or near their tips, which apparently is associated with coiling of the axonemes within them.

3. Paddle-type ciliated cells develop on the embryonic body proper and are always isolated from each other except at the anterior edges of the mantle. These cilia beat unidirectionally and asynchronously. The axis of the paddle is parallel to the cell surface on the return stroke, thus offering little resistance to the bending of the cilia, and is perpendicular to the direction of the power stroke, thereby increasing the effectiveness of the power stroke. Cells of this type appear on the mantle and head in patches, but not in areas to be covered by other tissues such as the mantle.

4. At or just before Stage 26, a third type of ciliated cell appears in rows on the mantle. The individual cilia are uniform in diameter and end in a tapered tip. The cilia on these cells beat in a metachronic wave synchronized among several cells aligned in rows. These lines of "uniform-type" ciliated cells are apparently very effective because the embryos begin to swim and tumble actively in the chorionic fluid and circulate it rapidly. This probably enhances respiration and prevents the embryo from sticking to the inner surface of the chorion.

5. A fourth type of beating ciliated cell, the "single-file" ciliated cell, appears on the head and ventral arms of the late Stage 28 or early Stage 29 embryo. The cilia of these cells are aligned in single file on the anterior-posterior axis and beat in a synchronized side-to-side wave. The function of these cells is unknown.

6. At hatching, the entire mantle epithelium degenerates and is sloughed off. Presumably, the epithelium of the head is also shed, but was unobserved by us.

LITERATURE CITED

Arnold, J. M., 1962. Mating behavior and social structure in *Loligo pealei*. *Biol. Bull.*, **123**: 53–57.

Arnold, J. M., 1965. Normal embryonic stages of the squid *Loligo pealei* (Lesueur). *Biol. Bull.*, **128**: 24–32.

Arnold, J. M., 1971. Cephalopods. Pp. 265–311 in G. Reverberi, Ed., in *Experimental embryology of marine and fresh water invertebrates*. North-Holland Publishing Co.

Arnold, J. M., and L. D. Williams-Arnold, 1976. The egg cortex problem as seen through the squid eye. *Am. Zool.*, **16**: 421–446.

Bergquist, P. R., C. R. Green, M. E. Sinclair, and H. S. Roberts, 1977. The morphology of cilia in sponge larvae. *Tissue & Cell*, **9**: 179–184.

Billett, F. S., and T. H. Courtenay, 1973. A stereoscan study of the origin of ciliated cells in the embryonic epidermis of *Ambystoma mexicanum*. *J. Embryol. Exp. Morphol.*, **29**: 549–558.

Grunz, H., A.-M. Multier-Lajous, R. Herbst, and G. Arkenberg, 1975. The differentiation of isolated amphibian ectoderm with or without treatment with an inductor. *Wilhelm Roux Arch. Entwickl.-Mech. Org.*, **178**: 277–284.

Kessel, R. G., H. W. Beams, and C. Y. Shih, 1974. The origin, distribution, and disappearance of surface cilia during embryonic development of *Rana pipiens* as revealed by scanning electron microscopy. *Am. J. Anat.*, **141**: 341–360.

Landstrom, U., 1977. On the differentiation of prospective ectoderm to a ciliated cell pattern in embryos of *Ambystoma mexicanum*. *J. Embryol. Exp. Morphol.*, **41**: 23–32.

Luft, J. H., 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.*, **9**: 409–414.

Smith, J. L., J. C. Osborn, and M. Stanisstreet, 1976. Scanning electron microscopy of lithium-induced exogastrulae of *Xenopus laevis*. *J. Embryol. Exp. Morphol.*, **36**: 513–522.

Sundermann-Meister, G., 1978. Ein neuertyp von cilienzellen in der Haut von spätembryonalen und juvenilen *Loligo vulgaris*. (Mollusca, Cephalopoda). *Zool. Jb. Anat.*, **99**: 493–499.